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Practitioner's Docket No. NEB-208-209-US ***PATENT***

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: GeorgeTzertzinis, GeorgeFeehery, Corinna Tuckey, ChristopherNoren, and LarryMcReynolds

Application No.: 10/622,240 Group No.: 1633
Filed: 07/18/2003 Examiner: Popa
For: Methods and Compositions Relating to Gene Silencing

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TRANSMITTAL OF APPEAL BRIEF
(PATENT APPLICATION--37 C.F.R. § 41.37)

1. Transmitted herewith, is the APPEAL BRIEF in this application, with respect to the Notice of Appeal filed on June 11, 2007.

2. STATUS OF APPLICANT

This application is on behalf of a small entity. A statement was already filed.

CERTIFICATION UNDER 37 C.F.R. §§ 1.8(a) and 1.10*

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Leslie Goldberg
Signature

Date: August 13, 2007

Leslie Goldberg
(type or print name of person certifying)

** Only the date of filing (' 1.6) will be the date used in a patent term adjustment calculation, although the date on any certificate of mailing or transmission under ' 1.8 continues to be taken into account in determining timeliness. See ' 1.703(f). Consider "Express Mail Post Office to Addressee" (' 1.10) or facsimile transmission (' 1.6(d)) for the reply to be accorded the earliest possible filing date for patent term adjustment calculations.*

3. FEE FOR FILING APPEAL BRIEF

Pursuant to 37 C.F.R. § 41.20(b)(2), the fee for filing the Appeal Brief is:

small entity	$250.00
Appeal Brief fee due	**$250.00**

4. EXTENSION OF TERM

The proceedings herein are for a patent application and the provisions of 37 C.F.R. § 1.136 apply.

Applicant believes that no extension of term is required. However, this conditional petition is being made to provide for the possibility that applicant has inadvertently overlooked the need for a petition and fee for extension of time.

5. TOTAL FEE DUE

The total fee due is:

Appeal brief fee	$250.00
Extension fee (if any)	$0.00
TOTAL FEE DUE	**$250.00**

6. FEE PAYMENT

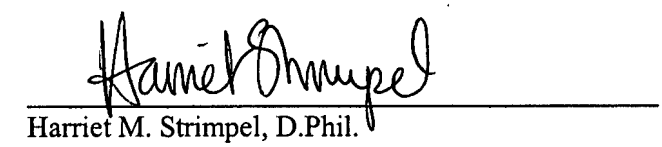
Attached is a check in the amount of $250.00.

A duplicate of this transmittal is attached.

7. FEE DEFICIENCY

If any additional extension and/or fee is required, and if any additional fee for claims is required, charge Deposit Account No. 14-0740.

Date: August 13, 2007



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PATENT
Attorney Docket No.: NEB-208/9-US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPELLANTS:	Tzertzinis *et al.*	CONFIRMATION NO.:	3580
APPLICATION NO.:	10/622,240	GROUP NO.:	1633
FILING DATE:	July 18, 2003	EXAMINER:	Popa, Ileana

TITLE: Methods and compositions relating to gene silencing

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P.O. Box 1450
Alexandria, VA 22313-1450

BRIEF IN SUPPORT OF APPEAL

Sir:

This is an appeal from the final rejection of claims 1, 2, 5-7, 9, 12-14, 16-18, 20 and 47, as amended by Appellants on June 11, 2007. The claims were finally rejected in a final Office action mailed from the USPTO on January 4, 2007. The amendment of June 11, 2007, was entered in an Advisory Action mailed from the USPTO on July 12, 2007, which maintained the rejection.

This Appeal Brief is submitted pursuant to the provisions of 37 C.F.R. § 41.37 and pursuant to the Notice of Appeal filed by Appellants pursuant to 37 C.F.R. § 41.31(a) on June 11, 2007. Appellants include herewith a check in the amount of $500.00 to cover the appeal brief submission fee pursuant to 37 C.F.R. § 41.20(b)(2) and 41.37(a)(2). Appellants believe no other fees are necessary for consideration of this paper. However, if a further fee is required, please consider this a conditional petition therefore and authorization to charge Deposit Account No. 50-1721.

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I. REAL PARTY IN INTEREST

The real party in interest is New England Biolabs, Inc. which owns the entire right, title, and interest in the instant application by virtue of assignment from the inventors of the instant application. The assignment from the inventors to New England Biolabs, Inc. was executed by George Tzertzinis, George Feehery, Corinna Tuckey, Christopher Noren and Larry McReynolds on July 17, 2003, and recorded in the U.S. Patent and Trademark Office on July 18, 2003, at Reel No. 014316, Frame No. 0124.

II. RELATED APPEALS AND INTERFERENCES

The Appellants, the Assignee, and the undersigned Attorney are not aware of any appeals, interferences, or judicial proceedings which may be related to, directly affect, or be directly affect by or have a bearing on the Board's decision in the pending appeal.

III. STATUS OF THE CLAIMS

Claims 1, 2, 5-7, 9, 12-14, 16-18, 20 and 47 stand rejected and remain pending. The rejection of these claims is appealed. Claims 8, 10, 15, 19, and 21-46 stand withdrawn from consideration. Claims 3, 4, and 11 have been canceled. The status of each claim is also set forth in the claims appendix following the argument section of this paper.

IV. STATUS OF AMENDMENTS

Appellants amended the pending claims on June 11, 2007. The amendments have been entered, as indicated in the Advisory Action mailed from the USPTO on July 12, 2007.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

Independent claim 1 is directed to a method of producing a plurality of overlapping double stranded (ds) RNA fragments of a size in the range of about 15-30 nucleotides. The method includes digesting a preparation of large double-stranded RNA in a reaction mixture containing a divalent transition metal cation and a prokaryotic RNaseIII. The ratio of enzyme to

substrate (w/w) is greater than or equal to about 0.25:1. The method includes producing the plurality of overlapping dsRNA fragments of a size in the range of about 15-30 nucleotides.

Support for the subject matter of independent claim 1 is found throughout the specification as originally filed, at least, for example, at page 5, lines 4-12 and 23-27; from page 20, line 23 through page 21, line 2; at page 26, lines 1-15; from page 32, line 3 through page 34, line 7; at page 36, lines 20-26; and at page 50, lines 18-21.

Independent claim 13 is directed to a purified set of double-stranded RNA fragments. The fragments include a plurality of overlapping fragments of a size in the range of about 15-30 nucleotides. The fragments in the set collectively represent a substantial portion of a sequence of one or more large double-stranded RNAs from which the fragments are derived by *in vitro* cleavage with a purified enzyme. One strand of each of the large double-stranded RNA has a sequence complementary to part or all of a target RNA.

Support for the subject matter of independent claim 13 is found throughout the specification as originally filed, at least, for example, from page 6, line 22, through page 7, line 2; at page 47, lines 9-26; at page 49, lines 21-26; at page 64, lines 19-21; at page 69, lines 20-27; and from page 74, line 16, through page 75, line 2.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

The sole ground of rejection remaining in this application is the rejection of claims 1, 2, 5-7, 9, 12-14, 16-18, 20, and 47 under 35 U.S.C. § 103(a) as allegedly unpatentable over U.S. Patent Application Publication No. US 2004/0014113 ("Yang") in view of Gross *et al.* (1987) Nucleic Acids Research 15:431-442 ("Gross").

Appellants request review of the ground of rejection on appeal.

VII. APPELLANTS' ARGUMENT

The rejection of the claims under 35 U.S.C. § 103 is inappropriate and should be reversed, because the differences between the prior art and the claimed subject matter are such

that one of ordinary skill in the art would not have had a reason to arrive at the claimed invention. The claimed invention differs from the prior art in ways that were not predictable, but that provide important advantages over the prior art. The invention therefore would not have been obvious to a person having ordinary skill in the art at the time the invention was made.

The importance of the differences between the prior art and the claimed invention, in view of the level of skill in the pertinent art, was made clear by the Supreme Court in *Graham v. John Deere Co. of Kansas City*, 383 U.S. 1 (1966). *Graham* set forth the framework that "define[s] the controlling inquiry" for applying the statutory language of 35 U.S.C. § 103. *KSR International Co.* v. *Teleflex*, 172 S.Ct. 1727 at 1734 (2007). Under *Graham*, determining the obviousness of a claimed invention requires determining the scope and contents of the prior art; ascertaining the differences between the prior art and the claims in issue; determining the level of skill in the pertinent art; and evaluating any evidence of secondary considerations. *See Graham*, 383 U.S. at 17-18.

A rejection of a claim as unpatentable requires first that the Examiner meet the initial burden of providing evidence and sound reasoning supporting the rejection. *See, e.g., In re Rijckaert*, 9 F.3d 1531, 1532 (Fed. Cir. 1993). "[R]ejections on obviousness grounds cannot be sustained by mere conclusory statements; instead, there must be some articulated reasoning with some rational underpinning to support the legal conclusion of obviousness." *In re Kahn*, 441 F.3d 977, 988 (Fed. Cir. 2006), *quoted in KSR*, 172 S.Ct. at 1741. If the initial burden is not met, a rejection cannot be affirmed.

Elements of a claimed invention working together in an unexpected and beneficial manner evidence the patentability of the invention. In an obviousness analysis, the operative question is often "whether the improvement is more than the predictable use of prior art elements according to their established functions." *In re Catan*, slip op. at 10 (Bd. Pat. App. & Inter. 2007), *quoting KSR*, 127 S.Ct. at 1740. Thus, for example, in *United States v. Adams*, 383 U.S. 39 (1966), "[t]he fact that the elements worked together in an unexpected and fruitful manner supported the conclusion that Adams's design was not obvious to those skilled in the art." *KSR*,

127 S.Ct. at 1740. Accordingly,

> [a]s is clear from cases such as *Adams*, a patent composed of several elements is not proved obvious merely by demonstrating that each of its elements was, independently, known in the prior art. Although common sense directs one to look with care at a patent application that claims as innovation the combination of two known devices according to their established functions, it can be important to identify a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed invention does. *KSR*, 127 S.Ct. at 1741.

The burden of identifying a reason that would have prompted a person of ordinary skill in the relevant field to combine the elements in the way the claimed invention does rests, in the first instance, with the Examiner. In the absence of such a reason, there is not even a *prima facie* case of obviousness. *See, e.g., Takeda Chemical Industries, Ltd. V. Alphapharm Pty., Ltd.*, 2007 WL 1839698 at *11 (Fed. Cir. 2007) ("The court properly concluded that Alphapharm did not make out a prima facie case of obviousness because Alphapharm failed to adduce evidence that compound b would have been selected as the lead compound and, even if that preliminary showing had been made, it failed to show that there existed a reason ... to perform the chemical modifications necessary to achieve the claimed compounds.").

The rejection of the claims under 35 U.S.C. § 103 cannot stand, because the Examiner has failed to establish even a *prima facie* case of unpatentability. The Examiner has not articulated any sound reason to combine and modify the teachings of the prior art to arrive at the claimed invention, and therefore has failed to establish a *prima facie* case of unpatentability. The Examiner has also failed to rebut Appellants' position that the cited references teach away from the claimed invention, again preventing the establishment of a *prima facie* case of unpatentability. The claimed invention does not involve the predictable use of prior art elements according to their established functions. The Examiner has not argued predictability and indeed made no findings under *Graham* regarding the level of skill in the pertinent art. As described in Appellants' specification and in the file history, Appellants' invention works in unexpected and fruitful ways compared to the prior art. For all of these reasons, Appellants' invention is patentable under 35 U.S.C. § 103 and the rejection must be reversed.

A. **Claim 1**

1. The Examiner has not articulated any sound reason to combine and modify the teachings of the prior art to arrive at the claimed invention.

Under *Graham*, determining the obviousness of a claimed invention requires determining the scope and contents of the prior art; ascertaining the differences between the prior art and the claims; determining the level of skill in the pertinent art; and evaluating any evidence of secondary considerations. *See Graham*, 383 U.S. at 17-18.

For the rejection, the Examiner relies on U.S. Patent Application Publication No. US 2004/0014113 ("Yang") and Gross *et al.* (1987) Nucleic Acids Research 15:431-442 ("Gross"). Yang teaches digestion of dsRNAs using *E. coli* RNaseIII. *See* Yang, abstract. Yang teaches that "[e]xhaustive cleavage of dsRNA by E. coli RNaseIII leads to duplex products averaging 12-15 bp in length." Yang, ¶ **[0015]**. Yang therefore teaches using "limited RNase III digestion" in an effort to obtain molecules of approximately 15-30 bp in length. *See* Yang, ¶¶ **[0015]**, **[0053]**. This limited digestion results in a smear on the agarose gel because of the wide range of sizes of fragments resulting from the partial digestion.[1] *See* Yang, Fig. 1B. Yang then

[1] Appellants and the Examiner have disagreed on certain aspects of the interpretation of Figure 1B of Yang. We do seem to agree, however, that lanes 1, 2, 4, 5, 7, and 8 of Figure 1B are smears of RNA of a variety of sizes. *See* the final Office action mailed January 4, 2007, at page 6 ("lanes 1, 2, 4, 5, 7, and 8 (i.e., the gradients of bands with different sizes or smears) ... (it is known in the art that RNA migrates as a smear on agarose gels)"). The Office action erroneously identifies the contents of those lanes as dsRNA "to be digested." *See id.*

Appellants appreciate that the poor quality of Figure 1B in the Yang U.S. patent application publication may have made its accurate interpretation more challenging. IDS reference CL contains what appears to be a much clearer version of Figure 1B. Appellants have included a copy of IDS reference CL in the Evidence Appendix at Tab A. IDS reference CL is a scientific publication whose authors include, among others, the Yang inventors.

In Figure 1B of IDS reference CL, the smears in lanes 1 and 2, above "1 min," are distant from the position marked with the arrow as indicating a size of 21-bp; the smears in lanes 4 and 5, above "3 min," begin to overlap the 21-bp position; and the smears in lanes 7 and 8, above "15 min," range from substantially greater to substantially smaller than the 21-bp position. The position in the final Office action mailed January 4, 2007, that these lanes represent dsRNA "to be digested" cannot be reconciled with the fact that the smears change substantially in size in the three panels corresponding to three different digestion times. The lanes necessarily correspond to the partial digestion products and evidence that, as one of ordinary skill in the art would predict from partial digestion, a wide range of sizes are produced.

selectively gel purifies the RNAs corresponding to approximately 21-30 bp from all of the other RNAs resulting from the limited digestion. *See* Yang, ¶ **[0053]**.

The differences between Yang and the invention of claim 1 are important. The claimed invention requires that the reaction mixture contain a divalent transition metal cation. The reaction mixture of Yang does not contain a divalent transition metal cation and Yang is silent about the possibility of including one. The claimed invention requires a ratio of enzyme to substrate (w/w) greater than or equal to about 0.25:1. Yang does not teach a ratio of enzyme to substrate greater than or equal to about 0.25:1. Indeed, the claimed ratio of enzyme to substrate is at least 20-fold higher than the ratio in Yang. Yang uses enzyme:substrate ratios of about 0.001:1. *See* Yang, ¶ **[0079]** ("100 μg of dsRNA were digested by 1 μg RNase III in a 200 μl reaction buffer"). The claimed invention requires the production of overlapping dsRNA fragments of a size in the range of about 15-30 nucleotides. Yang does not demonstrate the production of overlapping dsRNA fragments of a size in the range of about 15-30 nucleotides.

No sound reason has been articulated for changing the limited digestion of Yang by increasing the amount of enzyme by at least 20-fold and using a divalent transition metal cation, nor was there a sound basis for concluding that this would lead to a desirable method for producing overlapping dsRNA fragments of a size in the range of about 15-30 nucleotides. Indeed, to limit digestion resulting from RNaseIII , Yang et al. performed the obvious steps of limiting time of incubation, reducing the amount of enzyme and lowering the temperature of the reaction. Yang et al. did not suggest the need for further improvements of a different character and did not make specific suggestions of such improvements.

The rejection is also based in part on Gross *et al.* (1987) Nucleic Acids Research 15:431-442 ("Gross"). Gross teaches using RNAseIII to cleave a 141 nucleotide single stranded RNA molecule having three double stranded regions, none of which is longer than about 15 bp. *See* Gross, abstract and Figure 3. Three sites in the RNA molecule "are efficiently cleaved in the presence of magnesium^{2+} ions, two additional sites are cleaved in the presence of manganese^{2+} ions at low monovalent salt concentrations (<0.05M)." Gross, abstract; *see also* Gross, page 432 ("additional secondary sites are cut in the presence of manganese (Mn^{2+}) instead of magnesium

(Mg^{2+}) ions"). Gross does not relate to the production of a plurality of overlapping double stranded RNA fragments of a size in the range of about 15-30 nucleotides; does not teach digesting a preparation of large double-stranded RNA; and does not teach using a ratio of enzyme to substrate greater than or equal to about 0.25:1.

Even in view of Gross, one of ordinary skill in the art did not have a reason to modify the techniques of Yang in such a way as to result in the claimed invention. The Examiner's position is that Gross provides a motivation to use manganese in the digestion reaction of Yang:

> It would have been obvious to one of skill in the art, at the time the invention was made, to use manganese ... for a more efficient production of hsiRNAs. The motivation to do so is provided by Gross *et al.*, who teach that replacing magnesium with manganese promotes the cleavage of additional sites in the dsRNA (p.432, first paragraph). Office action of April 4, 2006, page 7.

This bears repeating: the Examiner's position is that one of ordinary skill in the art would want to replace magnesium with manganese to promote the cleavage of additional sites in the dsRNA.

The Examiner's position disregards the fact that Yang teaches the criticality of **limiting** the digestion. Yang teaches that exhaustive cleavage of dsRNA leads to duplex products averaging 12-15 bp in length that are unable to trigger an RNAi response. *See* Yang, ¶ **[0015]**. One of ordinary skill in the art would not have wanted to promote the cleavage of ***additional*** sites in the method of Yang, as that would only ***exacerbate*** the problems Yang was trying to address by **limiting** the digestion. *KSR*, recent Federal Circuit decisions and recent precedential decisions of the Board of Patent Appeals and Interferences all urge that the application of common sense is an essential part of any analysis under 35 U.S.C. § 103. *See, e.g., KSR*, 127 S.Ct. at 1742-1743; *see also DyStar Textilfarben GmbH & Co. Deutschland KG v. C.H. Patrick Co.*, 464 F.3d 1356, 1367 (Fed. Cir. 2006) ("Our suggestion test is in actuality quite flexible and not only permits, but *requires,* consideration of common knowledge and common sense"). Here, even under Yang's original conditions, a reaction that is permitted to proceed too far destroys the utility of the reaction products. Under these circumstances, common sense militates against promoting the cleavage of *additional* sites, rather than limiting the digestion as Yang taught to be necessary. Where Yang teaches limiting digestion, and Gross teaches increasing

digestion, the references themselves teach away from their combination and from the claimed invention.

Thus, the Examiner has provided no sound basis for modifying Yang to include a divalent transition metal cation.

The Examiner has also provided no sound basis for further modifying Yang to increase the amount of enzyme by at least 20 fold. Again, it is important to remember that Yang teaches the importance of limiting digestion. No rationale has been provided explaining why one of ordinary skill in the art would have increased the amount of enzyme by at least 20 fold despite the importance in Yang of limiting digestion. Further aggravating this deficiency in the rejection is that the claimed invention, in certain embodiments, requires both the presence of manganese ions described by Gross and the presence of at least 20 fold more enzyme than taught by Yang. Simultaneously making both changes would have seemed calculated to multiply the increases in a cleavage reaction that Yang sought to limit. The Examiner has not explained why one of ordinary skill in the art would have wanted to increase the enzyme by at least 20-fold in this context as this would have been expected to aggravate the problem rather than solve it. Instead, the Advisory Action of July 12, 2006, merely relies on an unsupported allegation that "[o]ne of skill in the art ... would recognize the necessity to [redefine] the optimum conditions when the reaction is performed in the presence of manganese." The Examiner does not explain why one of ordinary skill in the art would even explore conditions involving such a high ratio of enzyme to substrate based on reading the Yang et al and Gross et al references and ignores the fact that the claimed invention differs from the art in multiple ways, each of which is unexpected and even contraindicated when compared to the cited art .

Regarding the production of overlapping fragments, the Advisory Action of July 12, 2007, argues that producing overlapping fragments "is an inherent property of RNase III, which unspecifically cleaves the RNA." Gross, however, teaches that RNaseIII cleaves RNA at three specific, identified locations in the presence of magnesium and at an additional two specific, identified locations in the presence of manganese. *See* Gross, abstract and Figure 3. Thus, the cited art relied upon by the Examiner shows that RNaseIII, under some circumstances,

specifically cleaves RNA. The discovery that overlapping fragments could be produced, covering a substantial percentage of a large substrate dsRNA, by using the claimed conditions was the Appellants' discovery and was not evidenced in the art on which the rejection relies.

Because there was no sound reason to combine and modify the teachings of the prior art to arrive at the claimed invention, no *prima facie* case of unpatentability has been established and the rejection must be withdrawn.

2. The Examiner has failed to rebut Appellants' position that the cited references teach away from the claimed invention, again preventing the establishment of a *prima facie* case of unpatentability.

Appellants have explained above how Yang and Gross teach away from their combination and from the claimed invention.

The Examiner's response is found in the Advisory Action of July 12, 2007: "The argument that Gross et al. teach away from the instant invention because they disclose specific cleavage by RNase III is not found persuasive because Gross et al. teach that the secondary sites are identical to the primary sites recognized by the E. coli RNase III in the presence of magnesium."

The Examiner's response is inconsistent with the Examiner's other positions regarding the teachings of Gross and is inconsistent with the facts of Gross. Gross teaches that "two *additional* sites are cleaved in the presence of manganese $^{2+}$ ions" (emphasis added). Gross, abstract. "Additional secondary sites are cut in the presence of manganese (Mn^{2+}) instead of magnesium (Mg^{2+}) ions." Gross, p. 432. In an RNA molecule containing one primary RNaseIII site, Gross identified "two Mn^{2+} dependent secondary sites." Gross, p. 439. The Examiner's response is also inconsistent with the Office action of April 4, 2006, at page 7: "The motivation to do so is provided by Gross et al., who teach that replacing magnesium with manganese promotes the cleavage of additional sites in the dsRNA."

Because the Examiner has not effectively rebutted Applicants' position that the cited references teach away from the claimed invention, the rejection must be withdrawn.

Furthermore, the Advisory Action of July 12, 2007, argues that "Gross et al. teach that cleavage by E. coli RNase III can be rendered more efficient by exchanging magnesium with manganese." That, indeed, supports Appellants' position that the references teach away from the claimed invention: one of ordinary skill, at the time the invention was made would have predicted that if exchanging magnesium with manganese was to have an effect, it would be one deleterious to the key objective of limiting digestion. Thus, the references teach away from the claimed invention, preventing a conclusion that they render the invention unpatentable under 35 U.S.C. § 103.

3. The claimed invention does not involve the predictable use of prior art elements according to their established functions.

One of ordinary skill in the art, considering the teachings of Yang and Gross, would have predicted that the claimed invention, with its ratio of enzyme to substrate at least twenty times higher than the ratio in Yang and its incorporation of a divalent transition metal cation, would rapidly cleave a large dsRNA into fragments too small to be of interest.

Appellants discovery, however, is that the claimed invention permits the preparation of overlapping, useful dsRNA fragments of a defined size (15-30) bp. Appellants recognized the significant problems raised by the use of RNaseIII and a buffer containing magnesium and sought to improve the method of generating siRNA fragments. It was discovered by Appellants quite fortuitously and contrary to the teaching of the prior art (see page 21 of the present application) that the presence of divalent transition metal cations in the RNAse reaction mixture *in vitro* had a beneficial effect on generating dsRNA fragments of desired size from large dsRNA (observed as a sharp band in, for example, Figure 1 of the present application) corresponding to siRNA. Appellants completed an extensive analysis of the conditions for optimizing the reaction as reported in the above application. A number of significant advantages for this methodology were revealed. *See, e.g.,* page 22 of the present application. For example, it was possible to cleave substantially all large dsRNA and to generate RNA fragments less than about 50 bases

without degrading the fragments. Consequently, size fractionation was not required and mixtures of fragments for siRNA could be obtained simply and efficiently by ethanol precipitation only. *See* page 74, lines 23 of the present application. It was also found that the siRNA fragments obtained by the claimed method could be mapped to the substrate dsRNA so that a substantial portion (see page 28 of the present application) of the substrate sequence was represented. *See, e.g.,* Figures 4A and 4B of the present application.

Appellants claimed method therefore does not involve the predictable use of prior art elements according to their known functions.

In view of Appellants' unpredictable discovery; the lack of any sound, articulated reason why one of ordinary skill in the art, beginning from Yang, would end at the claimed invention; and the teachings of Yang and Gross that militate against modifying Yang in any way leading toward the claimed invention, the rejection of claim 1 must be reversed.

B. **Claims 2 and 9**

The rejection of claims 2 and 9 must be reversed for all of the reasons set forth above with respect to the rejection of claim 1.

Furthermore, the invention of claim 2 requires that the plurality of overlapping fragments be the product of "complete" digestion of the preparation of large double-stranded RNA.

The references cannot render the invention of claim 2 obvious, because Yang teaches that "exhaustive cleavage leads to duplex products averaging 12-15 bp in length" that are unable to trigger an RNAi response in mammalian cells. Yang, ¶ **[0015]**. Yang, in teaching away from "exhaustive cleavage," teaches away from "complete digestion" as claimed in claim 2.

Because the cited references teach away from the invention of claim 2, and because no reason has been proferred why one of ordinary skill in the art would have been inclined to conduct a complete digestion as claimed, the invention of claim 2 cannot be obvious.

C. **Claims 5-7**

The rejection of claims 5-7 must be reversed for all of the reasons set forth above with respect to the rejection of claim 1.

Furthermore, because claims 5-7 require that the transition metal cation be manganese, which is precisely the transition metal cation reported by Gross to promote *additional* cleavage events when used with RNAseIII, Appellants submit that one of ordinary skill in the art would have been particularly unlikely to choose to incorporate manganese as has been done in the method of claims 5-7. Accordingly, Appellants submit that the invention of claims 5-7 cannot be obvious.

D. **Claim 12**

The rejection of claim 12 must be reversed for all of the reasons set forth above with respect to the rejection of claim 1.

Furthermore, claim 12 relates to a method of silencing expression of a target gene. Based on the teachings in the art, one of ordinary skill in the art could not have reasonably expected that a digestion in accordance with the method of claim 1 would lead to products that should be used in a method of silencing expression of a target gene. Again, Appellants are reminded of the warning in Yang about exhaustive cleavage leading to "short dsRNA ... unable to trigger an RNAi response in mammalian cells." Yang, ¶ **[0015]**. Accordingly, claim 12 cannot be obvious in view of the cited references.

E. **Claims 13, 14, 16-18, 20 and 47**

The invention of claims 13, 14, 16-18, 20 and 47 relates to a purified set of double-stranded RNA fragments, comprising a plurality of overlapping fragments of a size in the range of about 15-30 nucleotides, the fragments in the set collectively representing a substantial portion of a sequence of one or more large double-stranded RNAs from which the fragments are derived by *in vitro* cleavage with a purified enzyme, one strand of each of the large double-stranded RNA having a sequence complementary to part or all of a target RNA.

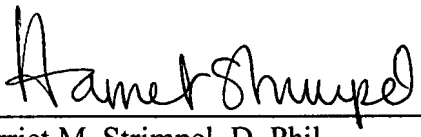
Neither Yang nor Gross suggests a purified set of dsRNA fragments that are overlapping and represent a substantial portion of the sequence of a target RNA.

The initial burden is on the Examiner to establish a *prima facie* case of unpatentability. Here, the Examiner has failed to explain why one of ordinary skill in the art would find the claimed invention predictable in view of the references. Gross suggests, in at least one context, that RNAseIII-mediated cleavage happens at defined positions. If cleavage sites were generally fixed positions, a person of ordinary skill in the art would not expect that cleavage products would be overlapping. Similarly, a person of ordinary skill in the art would not expect, even if overlapping fragments occasionally occurred, that they would collectively represent a substantial portion of a sequence of one or more large target RNA. The Examiner has presented no evidence that one of ordinary skill in the art would have expected to produce overlapping fragments collectively representing a substantial portion of a sequence of one or more large double-stranded RNAs.

The operative question, in the language of *Catan* and *KSR*, is "whether the improvement is more than the predictable use of prior art elements according to their established functions." *Catan*, slip op. at 10, *quoting KSR*, 127 S.Ct. at 1740. Here, the Examiner has failed to provide any evidence that the claimed invention was predictable in view of the art. Appellants discovered, despite the problems in the art, that Appellants could prepare the claimed overlapping dsRNA fragments collectively representing a substantial portion of a large sequence.

In view of the unpredictability in the art prior to Appellants' discovery, and the failure of the Examiner to establish a *prima facie* case of obviousness, the rejection of claims 13, 14, 16-18, 20 and 47 under 35 U.S.C. § 103 must be reversed.

Respectfully submitted,



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CLAIMS APPENDIX

1. (previously presented) A method of producing a plurality of overlapping double stranded (ds) RNA fragments of a size in the range of about 15-30 nucleotides, comprising:

(a) digesting a preparation of large double-stranded RNA in a reaction mixture containing a divalent transition metal cation and a prokaryotic RNaseIII wherein the ratio of enzyme to substrate (w/w) is greater than or equal to about 0.25:1; and

(b) producing the plurality of overlapping dsRNA fragments of a size in the range of about 15-30 nucleotides.

2. (previously presented) A method according to claim 1, wherein the plurality of overlapping fragments is the product of complete digestion of the preparation of large double-stranded RNA.

3-4 (cancelled)

5. (original) A method according to claim 1, wherein the transition metal cation is manganese.

6. (original) A method according to claim 5, wherein the reaction mixture contains manganese ions at a concentration in the range of about 5-10 mM.

7. (previously presented) A method according to claim 5, wherein the reaction mixture contains manganese ions at a concentration in the range of about 10-20 mM.

8. (withdrawn) A method according to claim 1, wherein the transition metal is selected from nickel, cobalt and cadmium.

9. (original) A method according to claim 2, wherein the complete digestion is achieved in less than 6 hours.

10. (withdrawn) A method according to claim 2, wherein the complete digestion is achieved in less than 2 hours.

11. (cancelled)

12. (previously presented) A method of silencing expression of a target gene, comprising:

introducing into a host cell, a plurality of fragments made according to claim 1, wherein the nucleotide sequence for each fragment has a sequence that is complementary to the target gene.

13. (previously presented) A purified set of double-stranded RNA fragments, comprising a plurality of overlapping fragments of a size in the range of about 15-30 nucleotides, the fragments in the set collectively representing a substantial portion of a sequence of one or more large double-stranded RNAs from which the fragments are derived by *in vitro* cleavage with a purified enzyme, one strand of each of the large double-stranded RNA having a sequence complementary to part or all of a target RNA.

14. (original) A set of fragments according to claim 13, wherein the substantial portion is greater than about 50% of the sequence of the large double-stranded RNA.

15. (withdrawn) A set of fragments according to claim 13, wherein the substantial portion is greater than about 65% of the sequence of the large double-stranded RNA.

16. (original) A set of fragments according to claim 13, wherein more than about 30% of the RNA fragments have a fragment size of about 18-25 base pairs.

17. (previously presented) A set of fragments according to claim 13, wherein at least one fragment and as many as 100% of fragments in the set are capable of causing cleaving the target RNA in a cell.

18. (previously presented) A set of fragments according to claim 17, wherein at least about 50% of the fragments are capable of causing cleavage of the RNA.

19. (withdrawn) A set of fragments according to claim 17, wherein at least about 75% of the fragments are capable of causing cleavage of the mRNA.

20. (original) A set of fragments according to claim 13, capable of RNA silencing *in vivo* when introduced into a eukaryotic cell.

21. (withdrawn) A method of creating a library of DNA clones from an hsiRNA mixture, each clone expressing one or more double-stranded RNA fragments from the hsiRNA mixture, the method comprising:

(a) denaturing the hsiRNA mixture to form a mixture of unpaired RNA strands;

(b) ligating to a 3' end of the unpaired RNA strands, a first single-stranded DNA primer and to a 5' end of the unpaired RNA strand, a second single-stranded DNA primer;

(c) reverse transcribing the chimeric DNA-RNA products of step (b) to form complementary DNA fragments; and

(d) inserting one or more DNA fragments into a vector to form the library of clones.

22. (withdrawn) A method according to claim 21, wherein step (c) further comprises performing a polymerase dependent amplification of the DNA fragments.

23. (withdrawn) A method according to claim 21, wherein the 5' end of the RNA strand in step (b) is dephosphorylated.

24. (withdrawn) A method according to claim 23, wherein the 3' end of the RNA strand in step (b) is a 3' hydroxyl end and where the first DNA primer has both a 5' and a 3' phosphate, the first primer being ligated to the 3' end prior to the second primer.

25. (withdrawn) A method according to claim 24, wherein the RNA strand ligated to the first primer of step (b) is phosphorylated and ligated to the second primer, wherein the second primer is non phosphorylated on the 3' ends.

26. (withdrawn) A method of creating a library of clones, each clone corresponding to one or more double-stranded RNA fragments from an hsiRNA mixture, the method comprising:

(a) denaturing the hsiRNA mixture to form a mixture of unpaired RNA strands;

(b) enzymatically removing the 5' phosphate from each strand in the mixture;

(c) ligating to the 3' hydroxyl end of each strand a DNA primer having both a 5' and a 3' phosphate;

(d) enzymatically phosphorylating the 5' end of the resulting species;

(e) ligating to the 5' phosphorylated end of each strand, a second DNA primer having nonphosphorylated 3' termini;

(f) reverse transcribing the chimeric DNA-RNA products of step (e) to form complementary DNA fragments; and

(g) inserting one or more DNA fragments into a vector to form the library of sequences.

27. (withdrawn) A method according to claim 26, wherein step (f) further comprises performing polymerase-dependent amplification of the DNA fragments.

28. (withdrawn) A method according to claim 26, wherein the vector is pUC19 or a Litmus vector.

29. (withdrawn) A kit for preparing an hsiRNA mixture, comprising: an preparation of RNAseIII, and an RNAse buffer containing manganese ions in the range of about 5 mM-100 mM and optionally reagents for synthesizing a large double-stranded RNA.

30. (withdrawn) A method of obtaining a large double-stranded RNA molecule, comprising;

(a) inserting a DNA fragment or library of DNA fragments encoding a double-stranded RNA into a vector having cloning sites flanked by opposing T7 promoters;

(b) performing in vitro or in vivo transcription; and

(c) obtaining the large double-stranded RNA molecule.

31. (withdrawn) A method of reducing expression of one or more target genes in a eukaryotic cell, comprising:

(a) introducing into the cell, a set of hsiRNA fragments according to claim 13, wherein the large dsRNA is complementary to all or part of a messenger RNA transcript of each of the target genes; and

(b) reducing the expression of the one or more target genes in the eukaryotic cell compared to expression of the genes in the eukaryotic cell absent the hsiRNA.

32. (withdrawn) A method of reducing expression of one or more target genes in a eukaryotic cell, comprising:

introducing into the cell, one or more DNA clones made according to claim 21 or 26, wherein the DNA clones express siRNA fragments suitable for reducing expression of the target eukaryotic cell compared to expression of the genes in the eukaryotic cell absent the DNA sequences.

33. (withdrawn) A method of claim 31 or 32, wherein the eukaryotic cell is present in a mammal such that reducing expression of the one or more target genes cause a phenotypic change.

34. (withdrawn) A method of claim 33, wherein the phenotypic change provides a treatment for a disease in the mammal.

35. (withdrawn) A method according to claim 34, wherein the phenotypic change is an enhancement of a desired characteristic in the mammal.

36. (withdrawn) A method according to claim 33, wherein the phenotypic change is diagnostic for a selected phenotype.

37. (withdrawn) A method according to claim 31 or 32, wherein the reduced expression of a gene is a tool for analyzing a biochemical pathway in which the gene product functions.

38. (withdrawn) A method according to claim 37, wherein the biochemical pathway may be further analyzed in combination with a diagnostic reagent.

39. (withdrawn) A method according to claim 38, wherein the diagnostic reagent is one or more antibodies.

40. (withdrawn) A method according to 31 or 32, wherein the eukaryotic cell is present in a non-human animal.

41. (withdrawn) A method according to claim 31 or 32, wherein the eukaryotic cell is a component of a transgenic animal is created from a fertilized oocyte containing the DNA sequence.

42. (withdrawn) A rapid discovery method for identifying an hsiRNA mixture which is capable of increased gene silencing of a target gene, comprising:

(a) synthesizing a plurality of large dsRNAs each large dsRNA having a sequence complementary to a segment of a target gene;

(b) digesting each of the large dsRNA with RNaseIII in the presence of a manganese ions to produce a corresponding hsiRNA mixture;

(c) introducing each hsiRNA mixture into a eukaryotic cell to determine whether gene silencing occurs; and

(d) determining which of the hsiRNA mixtures caused increased gene silencing.

43. (withdrawn) A method according to claim 42, wherein step (d) further comprises combining a first hsiRNA mixture with a second hsiRNA mixture for increasing gene silencing.

44. (withdrawn) A method according to claim 42, further comprising:

selecting individual siRNA fragments from hsiRNA mixtures and introducing the individual siRNA fragments into a eukaryotic cell to achieve desired gene silencing

45. (withdrawn) A method of identifying a sequence corresponding to an siRNA from a cleavage site in a mRNA, comprising:

(a) obtaining an hsiRNA mixture enzymatically;

(b) introducing the hsiRNA into a cell;

(c) extracting cleaved mRNA from the cell;

(d) determining the sequence of terminal nucleotides at the cleavage site of the siRNA cleaved mRNA; and

(e) identifying the siRNA sequence from the cleavage site sequence and neighboring nucleotides from the intact mRNA.

46. (withdrawn) A method according to claim 45, wherein the step of determining the sequence further comprises using labeled extension DNA primers.

47. (previously presented) A purified set of double-stranded RNA fragments according to claim 13, wherein the fragments bind specifically to mRNA to initiate cleavage of the mRNA.

EVIDENCE APPENDIX

Tab A	IDS Reference CL, Yang *et al.*, "Short RNA duplexes produced by hydrolysis with *Escherichia coli* RNase III mediate effective RNA interference in mammalian cells," Proc. Natl. Acad. Sci. 99: 9942-9947 (2002). (This publication was submitted to the USPTO in an Information Disclosure Statement mailed on February 9, 2004. The reference was received at the USPTO and was scanned into the Image File Wrapper, being accorded a filing date of February 11, 2004. As no action on the merits of the application had yet been taken, the Information Disclosure Statement , including IDS Reference CL, was admitted into the application on that day as a matter of right under 37 C.F.R. § 1.97(b)(3). The examiner initialed the Information Disclosure Statement on March 31, 2006, including the entry corresponding to IDS Reference CL, confirming that the reference had been admitted and considered.)

RELATED PROCEEDINGS APPENDIX

None.